

Does the menstrual cycle influence the sensitivity of vagally mediated baroreflexes?

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A B S T R A C T

The menstrual cycle provokes several physiological changes that could influence autonomic regulatory mechanisms. We studied the carotid–cardiac baroreflex in ten healthy young women on four occasions over the course of their menstrual cycles (days 0–8, 9–14, 15–20 and 21–25). We drew blood during each session for analysis of oestrogen, progesterone and noradrenaline (norepinephrine) levels, and assessed carotid–cardiac baroreflex function by analysing R-R interval responses to graded neck pressure sequences. Oestrogen levels followed a classical two-peak (cubic) response, with elevated levels on days 9–14 and 21–25 compared with days 0–8 and 15–20 ($P = 0.0032$), while progesterone levels increased exponentially from days 9–14 to days 21–25 ($P = 0.0063$). Noradrenaline levels increased from an average of 137 pg/ml during the first three measurement periods to 199 pg/ml during days 21–25 ($P = 0.0456$). Carotid–cardiac baroreflex gain and operational point were not statistically different at any of the time points during the menstrual cycle ($P \geq 0.18$). These findings are consistent with the notion that beat-to-beat vagal–cardiac regulation does not change over the course of the normal menstrual cycle.

INTRODUCTION

The Framingham study found that healthy young women experience symptoms of unexplained syncope more often than their male counterparts [1]. Although the mechanisms responsible for orthostatic hypotension are not fully understood, and vary among different clinical conditions, clues to understanding gender influences are beginning to emerge. Women pool more blood in their pelvic regions [2] and constrict their peripheral vasculature less [3,4] during orthostatic challenges than men. Although women who tend to have lower orthostatic tolerance than

men also have reduced carotid–cardiac baroreflex responses [5], a cause-and-effect relationship has not been proven.

Different phases of the normal human menstrual cycle are associated with variations in heart rate [6], arterial pressure [7], plasma volume [8], vascular tone [9], postural vasoconstriction [6] and plasma noradrenaline (norepinephrine) levels [10]. Because oestrogens are powerful vasodilators [11], increases in oestrogen levels during the menstrual cycle should set in motion a cascade of events driven, at least in part, by arterial baroreflexes. Although one study showed that integrated (aortic,

Key words: baroreflex, reproductive hormones, women.

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Report Documentation Page				Form Approved OMB No. 0704-0188	
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1. REPORT DATE 01 JUN 2002		2. REPORT TYPE N/A		3. DATES COVERED -	
4. TITLE AND SUBTITLE Does the menstrual cycle influence the sensitivity of vagally mediated baroreflexes?				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Cooke W. H., Ludwig D. A., Hogg P. S., Eckberg D. L., Convertino V. A.,				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) United States Army Institute of Surgical Research, JBSA Fort Sam Houston, TX 78234				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release, distribution unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT SAR	18. NUMBER OF PAGES 6	19a. NAME OF RESPONSIBLE PERSON
a. REPORT unclassified	b. ABSTRACT unclassified	c. THIS PAGE unclassified			

carotid and cardiopulmonary) sympathetic baroreflex sensitivity actually increases when oestrogen levels are high [12] (and possibly protects against orthostatic hypotension), the influence of the menstrual cycle on the isolated carotid–cardiac baroreflex is unknown. It remains possible that autonomic regulatory changes associated with the menstrual cycle underlie syncopal episodes in women.

To gain insight into the effects of the menstrual cycle on the isolated carotid–cardiac baroreflex, we rapidly changed carotid distending pressure with neck pressure and suction, and evaluated the resulting R-R interval responses, at different times during the normal menstrual cycle.

METHODS

Subjects

We studied ten healthy normotensive women with an average age ($\pm$ S.E.M.) of 25 ± 2.0 years (range 22–40 years). The study was conducted in accordance with the Declaration of Helsinki (1989) of the World Medical Association, and was approved by the human research committees of the Hunter Holmes McGuire Department of Veterans Affairs Medical Center and the Medical College of Virginia at Virginia Commonwealth University. Each subject gave written informed consent before participating. All subjects abstained from the use of caffeine, alcohol and tobacco during the study. None of the subjects had taken any regular medication, including oral contraceptives, during 1 year prior to the study period. All subjects reported regular 28–30-day menstrual cycles for at least 1 year prior to the study.

Baroreflex measurements

We applied sequential neck pressure and suction to modulate carotid baroreceptors, and recorded the resulting changes in R-R interval. During held expiration, a computer-controlled bellows increased neck pressure to approx. 40 mmHg for 5 s, and then decreased pressure by R-wave-triggered decrements of 15 mmHg, to approx. –65 mmHg. Stimuli were delivered during held expiration to minimize the influence of respiration on baroreflex responses. On each study day, each baroreflex sequence was repeated seven times for each subject, and the responses were averaged. Changes in R-R interval were plotted against carotid distending pressure (systolic pressure minus neck chamber pressure).

Recordings

We recorded the ECG, neck chamber pressure and respiration (abdominal bellows). We also measured

serum progesterone, oestrogen and noradrenaline levels. Reproductive hormone levels were determined with RIA techniques (Diagnostic Products Corp., Los Angeles, CA, U.S.A.), and noradrenaline levels were measured with HPLC.

Protocol

Data were obtained from study subjects on four separate occasions, between days 0–8, 9–14, 15–20 and 21–25 of their menstrual cycles (the day menses began was designated day 0). At the beginning of each data collection period, sphygmomanometric blood pressures were recorded with the subject in the supine position. Subjects were then fitted with ECG electrodes and a respiratory bellows. A 21-gauge butterfly needle was inserted into an antecubital vein for blood sampling. Subjects then rested quietly in the supine position for 20 min. At the end of the 20 min period, blood samples were drawn for hormone analyses, and the butterfly needle was withdrawn. The neck chamber was then applied, and baroreflex responses were measured.

R-R interval responses

Changes in R-R interval were used as surrogates for directly recorded vagal–cardiac nerve traffic [13]. We derived a maximal slope and operational point from R-R interval responses. The maximal slope was calculated from linear regression analysis of each set of three consecutive pairs of data on the linear portion of the stimulus–response relationship. The operational point was derived as:

$$\{[(\text{R-R interval at 0 mmHg neck pressure}) - (\text{minimum R-R interval})] / \text{R-R interval range}\} \times 100.$$

Statistics

The statistical model for all analyses was a four-period (menstrual cycle category), within-subject, repeated-measures ANOVA. Since subjects were studied serially over their menstrual cycles and not randomly, appropriate single degree of freedom tests with subsequent exact error term decomposition were generated to account for the time dependency in the data. This was done using either orthogonal polynomials (i.e. trend analysis) or orthogonal contrasts. The results of statistical tests are given as exact *P* values, and reflect the estimated probability of obtaining the observed effect in a noise-only system. Standard errors are given as raw (i.e. time-specific) standard errors unadjusted for subject-to-subject variation or the autocorrelation structure of the serial time measurements. Three of the subjects had single missing values for all hormones. Least-squared estimation was used to correct for these missing values,

resulting in slightly fewer error degrees of freedom available for tests involving hormonal comparisons. All statistical computation was done using JMP Statistical Discovery Software (SAS Institute, Cary, NC, U.S.A.).

RESULTS

Reproductive hormones

Figure 1 shows oestrogen and progesterone concentrations for each subject across the four menstrual cycle time categories. For oestrogen, all subjects demonstrated a cubic trend, with two primary peaks occurring on days 9–14 and 21–25 of the menstrual cycle. For progesterone, the overall trend was quadratic, since only trace levels of progesterone were present up to 15 days. After day 15 the trend was linear, with peak values occurring during days 21–25. The average statistical trends estimated from orthogonal polynomials for both oestrogen [cubic, $F(1,6) = 22.45$, $P = 0.0032$] and progesterone [quadratic, $F(1,6) = 16.88$, $P = 0.0063$] have

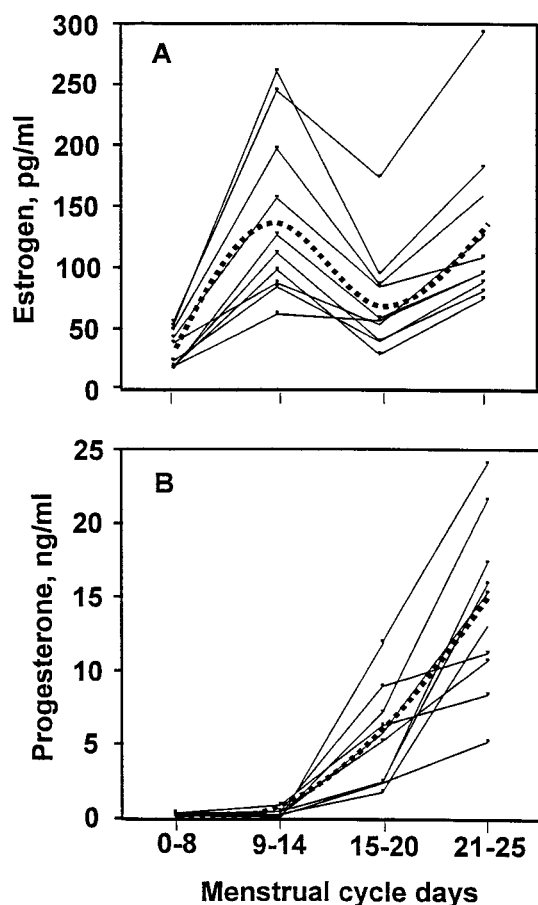


Figure 1 Oestrogen (A) and progesterone (B) concentrations for all subjects during the menstrual cycle

Solid lines connect data points from individual subjects; broken lines show the trend functions.

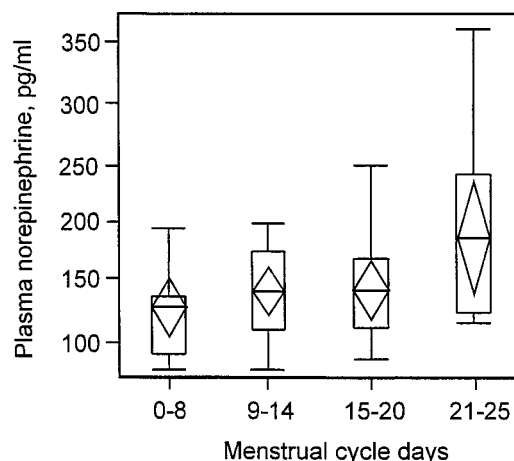


Figure 2 Mean noradrenaline (norepinephrine) concentrations during the menstrual cycle

Box-and-Whisker plots represent variation and distribution properties of the individual observations. Variations in mean values (± 2 S.E.M.s) are represented by diamonds (approximate 95% confidence intervals).

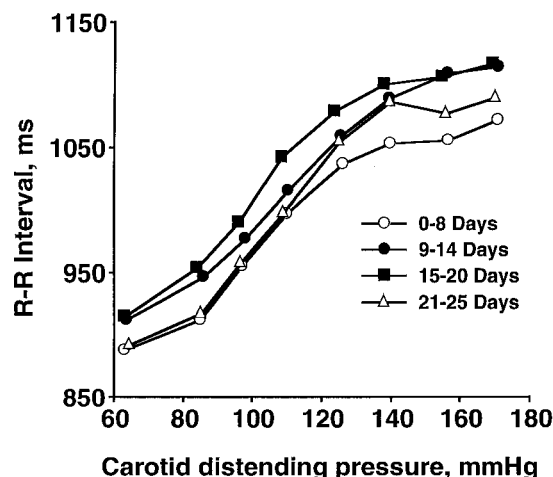


Figure 3 Mean carotid-cardiac baroreflex responses to neck pressure and suction during the menstrual cycle

been superimposed over the individual data points in Figure 1.

Noradrenaline

Figure 2 shows noradrenaline concentrations across the four menstrual cycle categories. Although the general trend for noradrenaline was to increase, virtually all of the increase occurred in the last 5 days of the menstrual cycle. Analysis of noradrenaline concentrations in the three menstrual cycle categories prior to day 21 indicated no statistical differences [$F(2,6) = 0.110$, $P = 0.8979$], while a comparison of the last 5 days of the cycle with the average of the first 20 days showed an increase of 62 pg/ml [$F(1,6) = 6.32$, $P = 0.0456$].

Table 1 Heart rate, arterial pressure and carotid–cardiac baroreflex responses during different phases of the normal menstrual cycle

Values are means $\pm$ S.E.M. ($n = 10$). Baroreflex responses are derived values from the neck pressure sequences (seven trials at each phase of the menstrual cycle); heart rate and arterial pressure were derived at baseline, before the application of neck pressure and suction.

Variable	Days 0–8	Days 9–14	Days 15–20	Days 21–25
Heart rate (beats/min)	64 $\pm$ 3.0	62 $\pm$ 2.0	61 $\pm$ 2.0	63 $\pm$ 2.0
Systolic pressure (mmHg)	103 $\pm$ 2.0	104 $\pm$ 2.0	103 $\pm$ 2.0	104 $\pm$ 2.0
Diastolic pressure (mmHg)	65 $\pm$ 2.0	63 $\pm$ 1.0	64 $\pm$ 1.0	63 $\pm$ 2.0
Baroreflex slope (ms/mmHg)	4.0 $\pm$ 0.4	3.8 $\pm$ 0.5	5.1 $\pm$ 1.0	4.2 $\pm$ 0.4
Operational point (%)	36 $\pm$ 6.0	35 $\pm$ 7.0	37 $\pm$ 5.0	31 $\pm$ 6.0

Baroreflex responses

Figure 3 shows average carotid–cardiac baroreflex response relationships for each of the four menstrual cycle time categories. We observed no statistically discernible differences in maximum slope, operational point, baseline heart rate or baseline arterial pressure among the different menstrual cycle day categories [$F(3,27) \leq 1.73$, $P \geq 0.18$]. Baroreflex data from the various menstrual cycle categories are listed with arterial pressures and baseline heart rates in Table 1.

DISCUSSION

We measured carotid–cardiac baroreflex responses of healthy female subjects to a series of stair-step neck pressure sequences delivered at different stages of the menstrual cycle. Levels of reproductive hormones during the menstrual cycle followed classical patterns [14], with little variation among subjects. Our primary finding is that, although levels of reproductive hormones and plasma noradrenaline fluctuate, carotid–cardiac baroreflex gain remains constant during the normal menstrual cycle. Our results argue against the notion that changes associated with the menstrual cycle may cause orthostatic hypotension in women by decreasing the responsiveness of the carotid–cardiac baroreflex.

To our knowledge, the present study is the first to investigate the influence of the human menstrual cycle on the isolated carotid–cardiac baroreflex. Our results indicate that the reflex is stable and reproducible throughout the menstrual cycle, and agree with those from other studies reporting integrated baroreflex responses. Integrated vagal baroreflex responses provoked using the Oxford method do not differ in young women compared in their early follicular and mid-luteal phases [12], or in older, postmenopausal women after hormone replacement therapy [15]. Integrated vagal baroreflex responses assessed in the frequency domain with cross-spectral techniques similarly are not different during different phases of the menstrual cycle [16]. Although women who

suffer episodes of orthostatic intolerance have blunted carotid–cardiac reflex responses in comparison with men [5], the present study and those Minson et al. [12] and Hunt et al. [15] strongly suggest that the menstrual cycle does not affect vagal baroreflex gain.

However, studies performed on both pregnant women and animals suggest that chronic exposure to elevated levels of reproductive hormones may compromise baroreflex function. Blake et al. [17] and Silver et al. [18] reported reductions of baroreflex sensitivity of 50% and 40% in pregnant, normotensive women. Although baroreflex sensitivity is not altered by chronic oestrogen treatment in non-pregnant rabbits [19], cardiovagal baroreflex sensitivity decreases in non-pregnant young women taking oral contraceptives [20]. Further research is required to determine whether short-term compared with long-term exposure to elevated levels of reproductive hormones differentially affects the arterial baroreflex.

Factors other than circulating levels of reproductive hormones could potentially impact on carotid–cardiac baroreflex responsiveness. For example, plasma noradrenaline levels [10], and presumably muscle sympathetic nerve activity [21], fluctuate during the menstrual cycle. We observed that the normal menstrual cycle in our subjects provoked subtle elevations of plasma noradrenaline similar to those reported previously [10]. Chronic increases in sympathetic nervous activity in states such as hypertension [3] and heart failure [21] are associated with decreases in vagal–cardiac nerve activity; however, before our investigation, it was not known whether increases in sympathetic activity associated with the menstrual cycle induced comparable reciprocal changes in vagal control. Airaksinen et al. [22] showed that intravenous infusion of noradrenaline caused uncoupling of the usual relationship between arterial pressure and R-R interval, suggesting that high levels of sympathetic nervous activity or of plasma noradrenaline may override or inhibit vagal–cardiac reflex responses. If elevated sympathetic activity opposes vagally mediated responses, one might expect a decrease in carotid–cardiac baroreflex responses from days 0–8 (when plasma nor-

adrenaline levels are low) to days 21–25 (when levels are high). However, Minson et al. [12] recently showed that hormonally induced increases in noradrenaline levels during the luteal phase of the menstrual cycle (when oestrogen levels are high) do not affect the heart rate response during the infusion of vasoactive drugs, suggesting that cardiac vagal control is not influenced by elevated sympathetic nervous activity associated with the normal menstrual cycle. Our data are consistent with the observations of Minson and co-workers [12], since we observed no alterations in the direct measurement of the isolated, vagally mediated carotid–cardiac baroreflex response in the presence of a 50% elevation in circulating plasma noradrenaline levels that occurred at the end of the menstrual cycle in our subjects.

Other factors that we did not measure could potentially alter carotid–cardiac baroreflex function. First, plasma volume changes throughout the menstrual cycle, and these changes could influence autonomic regulatory mechanisms and plasma noradrenaline levels. However, acute or chronic expansion (as much as 10%) or reduction (as much as 15%) of blood volume using fluid loading, diuretics or exercise training does not alter the carotid–cardiac baroreflex [23,24]. Blood volume fluctuations of the order of 10–15% have been reported during the normal menstrual cycle [8]. Secondly, oestrogens have been shown to decrease vascular responses to contractile agents in both dogs [25] and rats [26]. Decreased vascular contractility due elevated oestrogen levels could be mediated by nitric oxide, levels of which were found to be 100% greater in women at mid-cycle compared with during menstruation [27]. It is possible that the fluctuations in plasma noradrenaline concentration we observed were modulated by fluctuating reproductive hormones levels, but it is as likely that plasma noradrenaline levels increased in response to nitric oxide-mediated vasodilation through baroreflex feedback. In fact, the separate influences of noradrenaline and nitric oxide could conceivably serve to maintain carotid–cardiac function at normal levels through reciprocal influences on vascular smooth muscle. Our data do not allow for speculation into the effects of nitric oxide-mediated vasodilation on plasma noradrenaline levels or carotid–cardiac baroreflex responses, but serve to suggest areas of future research.

Our results should be considered in the light of at least two limitations. First, we used a physiological elevation of noradrenaline during the normal menstrual cycle to speculate that heightened levels of sympathetic nervous activity may inhibit vagal–cardiac control. Clearly, we are observing the influence of noradrenaline, reproductive hormones and other associated factors that we did not measure. Secondly, during days 9–14 of the cycle, oestrogen levels were high and progesterone and noradrenaline levels were low. On days 21–25, oestrogen, progesterone and noradrenaline levels were all high.

Although we are not aware of any evidence that progesterone influences vagal–cardiac reflex responses, we recognize that we did not account for possible influences of elevated progesterone levels during days 21–25. On the other hand, the absence of any effect on the carotid–cardiac baroreflex of fluctuating reproductive hormone levels, regardless of whether oestrogen and progesterone were changing in parallel across the menstrual cycle, argues against a confounding influence of progesterone.

In summary, our data show that carotid–cardiac baroreflex gain does not change across the normal human menstrual cycle. Although the mechanisms underlying syncopal episodes in women (compared with men) include blunted carotid–cardiac reflex responsiveness, our results suggest that such reductions of carotid–cardiac reflexes are not linked to a particular phase of the menstrual cycle.

ACKNOWLEDGMENTS

We thank Janice Meck (Johnson Space Center, Houston, TX, U.S.A.) for her helpful comments and suggestions during the preparation of this paper. This work was supported by Faculty Research Fellowships (W.H.C. and D.A.L.) through the US Army Institute of Surgical Research (V.A.C.) under the auspices of the US Army Research Office Scientific Services Program administered by Battelle (Delivery Order 710; Contract No. DAAH04-96-8806), a Scientist Development Grant from the American Heart Association (W.H.C.; 0030203N), and a Faculty Scholarship Grant from Michigan Technological University (W.H.C.).

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Received 8 November 2001; accepted 28 February 2002